Received: 31 October 2017 | Accepted: 20 January 2018

DOI: 10.1002/joa3.12042

CASE REPORT

WILEY Journal of Arrhythmia Open Access

Detection of a new KCNQ1 frameshift mutation associated with Jervell and Lange-Nielsen syndrome in 2 Iranian families

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Funding information
Pasteur Institute of Iran, Grant/Award Number: 824

Abstract

Jervell-Lange Nielsen syndrome (JLNS) with autosomal recessive inheritance is a congenital cardiovascular disorder characterized by prolongation of QT interval on the ECG and deafness. We have performed molecular investigation by haplotype analysis and DNA Sanger sequencing in 2 unrelated Iranian families with a history of syncope. Mutational screening of *KCNQ1* gene revealed the novel homozygous frameshift mutation c.733-734delGG (p.G245Rfs*39) in 2 obviously unrelated cases of JLNS which is probably a founder mutation in Iran. The novel mutation detected in this study is the first time reported among Iranian population and will be beneficial in the tribe and region-specific cascade screening of LQTS in Iran.

KEYWORDS
founder mutation, Iran, Jervell and Lange-Nielsen syndrome, *KCNQ1*, long-QT syndrome

1 | INTRODUCTION

The congenital long-QT syndrome (LQTS), a prolongation of the QT interval at electrocardiogram (ECG), is a severe cardiac arrhythmia.[1] Inherited LQTS have been characterized in 2 forms: autosomal-dominant Romano-Ward syndrome (RWS) and Jervell-Lange Nielsen syndrome (JLNS). Romano-Ward syndrome (MIM# 192500) is the most common form of inherited LQTS, with an estimated incidence of 1:5000-1:10 000 live births. JLNS (MIM# 220400) is less prevalent (1:50.000). In these patients, LQTS is associated with congenital sensorineural deafness, and the pattern of inheritance is autosomal recessive.[2]

In 1993, Schwartz et al[3] developed the diagnostic criteria for clinical diagnosis of LQTS that is essential to identify asymptomatic carriers. LQTS is characterized by some features including extended QT interval and T-wave alteration in ECG, syncope, ventricular tachycardia (VT), torsade de points (TdP) and an increased risk of sudden death due to TdP or VT.[4] So far, a panel of sixteen candidate genes has been known that heterozygous mutations in them cause Romano-Ward syndrome and mainly 2 genes that

homozygous or compound heterozygous mutations in them are responsible for Jervell and Lange-Nielsen syndrome.[5,6] Mutations that lead to defects in cardiac rapidly (I_{Kr}) and slowly (I_{Ks}) activating delayed rectifier potassium channels and sodium channel (I_{Na}) can cause Romano-Ward syndrome while Jervell and Lange-Nielsen syndrome solely is caused by defects in I_{Ks} that is crucial electrical part of cardiac action potential as well as indispensable for myocardial repolarization.[7,8]

The most commonly mutated gene among causative genes for LQTS is *KCNQ1*.[9-11] It encodes the subunit of the slow delayed rectifier potassium channel. *KCNQ1* has 6 transmembrane segments, and combination of normal and mutant subunits results in the incomplete formation of the channel and consequently loss of channel function.[7]

Based on clinical and molecular findings and exact diagnosis, treatment of JLNS will be managed. In this study, we managed to discover a homozygous frameshift deletion in *KCNQ1* gene in 2 Iranian JLNS patients for the first time.

2 | MATERIALS

2.1 | Patients

Two unrelated LQTS patients were referred to the emergency unit at the Rajaei Cardiovascular Medical and Research Center, Tehran, Iran, for further clinical evaluations:

2.1.1 | Patient 1

The first proband was a 5-year-old boy who was referred to our center due to syncope. The medical history showed recurrent bradycardia during the fetal period, syncope, and cochlear implantation at 4 for management of sensory-neural hearing loss. There was no family history of syncope, faint or sudden death in 5 generations. In the paraclinical studies, the level of electrolytes and hormone analysis was in normal range. Resting 12-lead electrocardiogram (ECG) displayed a manifestly prolonged QTc interval of more than 600 ms (corrected by Bazett's formula) and T-wave alternant (Figure 1A). Also, a structurally normal heart was detected by echocardiography report. The ECG of his asymptomatic parents showed a totally normal electrocardiogram. Propranolol with the dose of 3 mg/kg/d, divided 3 times a day, was started for the patient. An endocardial single-chamber implantable cardioverter defibrillator (ICD) was implanted to prevent any life-threatening events.

2.1.2 | Patient 2

The proband was a 3-year girl with recurrent syncopes and congenital neurosensory deafness. She had a QTc interval of 540-560 ms (corrected by Bazett's formula) and T-wave alternant on V1-V4 (Figure 1B). She had a history of fainting, while she was in the bath and during exercise. Her first syncope episode had occurred at 19 months of age. Physical and neurological examinations were normal except for hearing impairment, and she did not have an electrolyte imbalance. At 15 months of age, she received a cochlear implant. Echocardiography showed a structurally normal heart. 2 mg/kg/d beta-blocker treatment (maximum dose of tolerate by this patient) was started divided 3 times per day. Her parents were asymptomatic, and their QTc intervals were in normal range. There were no other siblings, and members of the extended family were not available for study.

3 | GENETIC STUDY

After obtaining written informed consent, blood samples of the patients and other family members were collected and genomic DNA was extracted by salting-out method.[12] At first, for haplotype analysis, linked short tandem repeat (STR) markers surrounding the *KCNQ1* gene were used. All STRs were selected among tetra-nucleotide repeat markers and were unique. Marker D11SI was an intragenic marker, while the other markers were selected by a maximum physical distance of less than 1.4 Mb at the upstream (D11SU0.6) or downstream (D11SD13.6, D11SD8.3) of the gene. Detecting the size of the repeats was performed by ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Then, primer pairs for all coding exons and exon-intron boundaries and untranslated regions of *KCNQ1* [NM_000218] gene were designed by primer 3 Online and gene runner software. All of the *KCNQ1* gene exons were amplified by PCR, and the amplicons were directly sequenced by Sanger sequencing chain termination method on ABI 3130XL Genetic Analyzer by Kawsar Biotech Co. (KBC, Tehran, Iran).[13] The results of Sanger sequencing were analyzed and compared against the RefSeq genomic accession numbers (NG_008935.1), and mutation database (http://www.genomed.org/lovd2/home.php) was used for evaluating the variants.

4 | RESULTS

Haplotype analysis by 6 linked STR markers surrounding the *KCNQ1* gene showed the disease in the family is linked to the *KCNQ1* gene. Sanger sequencing of the 2 probands showed a novel homozygous mutation, c.733-734delGG (p.G245Rfs*39), in exon 5 of *KCNQ1* gene (ClinVar accession number: SCV000584046). The 2-bp deletion results in a frameshift deletion (G245Rfs*39) in the cytoplasmic loop (C loop) between the transmembrane region (S4-S5 C loop) of KvLQT1 (*KCNQ1* protein) (Figure 2). This mutation introduces 38 novel amino acids after codon 244 (glycine 245 as the first affected amino acid has altered into Arginine) and premature stop codon at 283 that resulted in a truncated protein. This mutation was also found in heterozygous form in the parents of 2 probands (Figure 3C).

Short tandem repeat markers help us demonstrating the same frameshift *KCNQ1* mutation in 2 obviously unrelated families derives from a unique origin (founder mutation) (Figure 3).

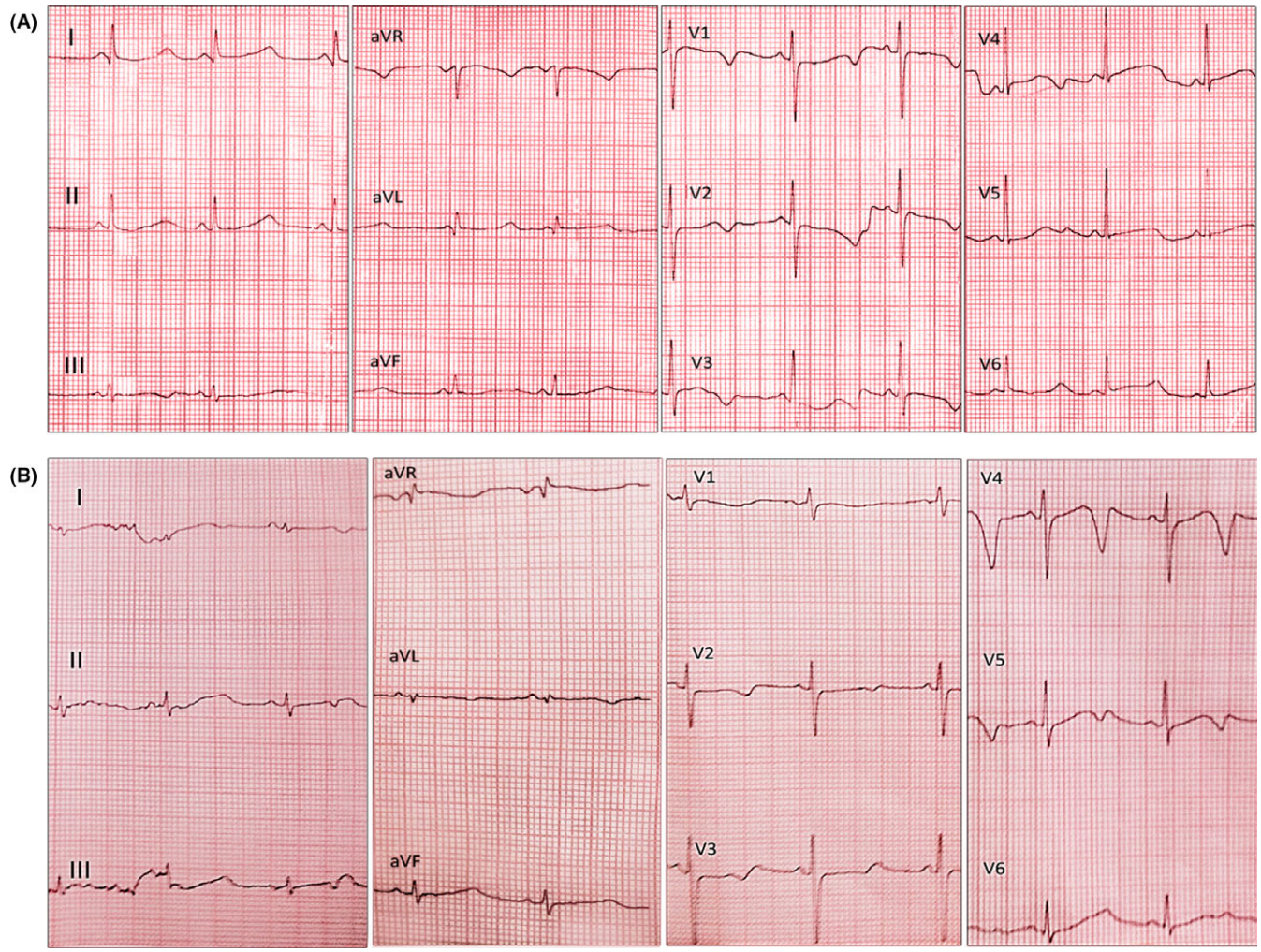

FIGURE 1 ECG of the patients. A, The 12-lead electrocardiogram of the patient A at 5 years of age demonstrating prolonged QTc interval (QTc>600 ms, heart rate: 62 beats/min) and T-wave alternancy. B, The 12-lead electrocardiogram of the patient B at 3 years of age demonstrating prolonged QTc interval; QTc>520 ms, heart rate: 72 beats/min (with a paper speed of 25 mm/s and 10 mm/mV at 20 Hz)

5 | DISCUSSION

Long-QT syndrome includes a variety of diseases caused by mutations in cardiac ion channels. Among the genes encoding ion channels of the heart, homozygous or compound heterozygous mutations in *KCNQ1* and *KCNE1* genes are responsible for the recessive type of LQTS known as JLNS. These 2 genes encode subunits responsible for a voltage-gated potassium channel formation, as the potassium current is the fundamental part of the cardiac repolarization and normal function of inner ear cells. In addition, it has an essential role to speed up the activating potassium current (IKs).[14,15] Mutations in either the *KCNQ1* or *KCNE1* genes affect potassium transport in the inner ear and cardiac muscle, causing deafness and an irregular heart rhythm.[16]

So far, 21 different homozygous or compound heterozygous mutations in *KCNQ1* gene and 3 homozygous or compound heterozygous mutations in *KCNE1* gene in JLNS patients have been characterized according to the Human Genome Database (HGMD), http://www.hgmd.cf.ac.uk/ac/index.php).[17]

In this study, we have identified a homozygous frameshift mutation in exon 5 (c.733-734delGG) of the *KCNQ1* gene in 2 nonconsanguineous families with JLNS among an Iranian cohort of LQTS patients. The mutation has not been reported previously in HGMD and 1000 genomes databases. This mutation occurs in the cytoplasmic loop(C loop) between the transmembrane regions (S4-S5 C loop) of *KCNQ1*. As C loops are critical in modifying the function of voltage-gated potassium channels, changing the C loop residues by mutations in this area of *KCNQ1* gene, effects on the voltage dependence of channel activation, leads to increased risk for lethal cardiac events.[18] Although functional assays of mutation were not conducted in these patients, however frameshift mutations, with an estimated predicted value (EPV) of 99%, were expected to have a pathogenic effect.[19]

Electrophysiological study revealed that mutations in the S4-S5 linker, W248R, and E261K, changed the voltage dependence of *KCNQ1* channels and caused deactivation of *KCNQ1*.[20] It has been reported that missense mutations in the S4-S5 linker, V254M, led to

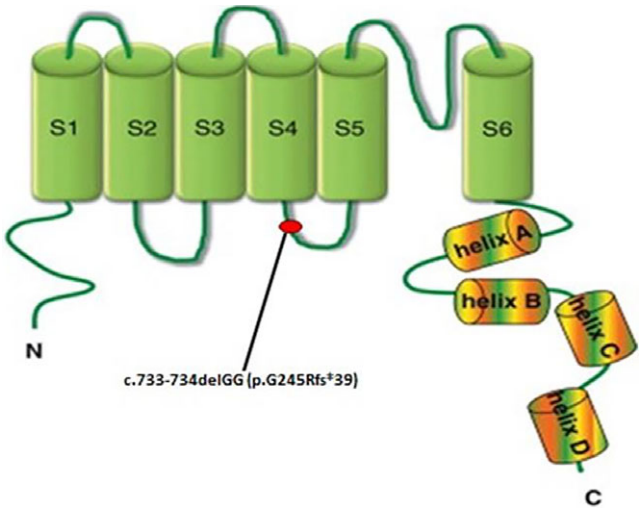

FIGURE 2 Scheme depicting *KCNQ1* mutation location (*Curr Opin Pharmacol* 2014;15:74-82)[30]

loss of function; however, mutant subunits along with the wild-type *KCNQ1* expedited the ratio of channel activation.[21]Therefore, as Sanguinetti et al[22] described, such as *KCNH2*, missense mutations in the S4-S5 linker of *KCNQ1* can either slack or expedite channel activation.[20] Moreover, other functional assay demonstrated that mutations in C loops of *KCNQ1* gene, in the absence[23] and presence of wild-type subunits, significantly impressed adrenergic channel regulation.[24] As JLNS patients tend to have mutations that cause frameshifts[25] and/or protein truncation[26] and it was suggested that truncating mutations result in loss of function,[27] in this report, support for this concept is provided. In both families, a frameshift mutation is present, with the truncating mutation leading to QT prolongation in affected individuals. In 2006, one report presented the guidelines that suggested an experimental β-blockers therapy in all LQTS patients,[28] meanwhile another study proved the effect of beta-blockers on affected individuals who have mutations in the C loops collated with mutations in another location that can decline the risk of lethal cardiac incidents.[24] According to previous studies,[6] patients with JLNS carrying *KCNQ1* mutations are at particularly high risk, and therefore, special aids, including beta-blocker therapy, may be supposed necessary because of the high recurrence rate of fatal arrhythmia. Both of our cases were treated with propranolol or in one instance ICD. During 1 year using propranolol therapy follow-up of these patients, any cardiac events were recorded. Unfortunately, although Left cardiac sympathetic denervation (LCSD) helps to reduce life-treating events in such cases, but we did not do it nowadays in our centers.

G245Rfs*39 mutation in LQTS patients has not been previously reported and appears to be a novel mutation. This mutation deletes 2 nucleotide GG between nucleotide position 733 and 734, resulted in a premature stop codon at position 283 that lead to the production of a truncated protein containing a total of 282 amino acids which its size is less than half of normal protein. Parents of 2 patients are heterozygous for the mutation (Figure 2C). They are asymptomatic, and ECG from the parents showed QTcs within normal limits. The 2 patients analyzed in this report are not resourcefully linked, haplotype analysis by linked STR markers surrounding the *KCNQ1* gene, demonstrating that the source of the mutation in both patients is the same. In addition, according to the previous statement,[29] in such a huge endogamous community in which consanguineous marriages are common, we would expect the same mutation in 2 apparently unrelated families originate from a unique source and could be a founder mutation. Both of families are from the southwestern of Iran (Khuzestan Province).

In summary, in the current study for the first time, we have identified a novel homozygous frameshift mutation in the *KCNQ1* gene in 2 Iranian patients with JLNS which expected to cause consequential structural changes in the encoded potassium channel subunit, and regards to ACMG, this finding is likely to severely diminish or abolish channel function. Based on the results of our study and previous studies, it is imperative to perform proper genetic analysis to confirm the diagnosis in order to select the optimal method for clinical care of the patient.

ACKNOWLEDGEMENTS

This work was supported by Pasteur Institute of Iran (grant number 824 partly and the Ph.D. grant from education office, Pasteur

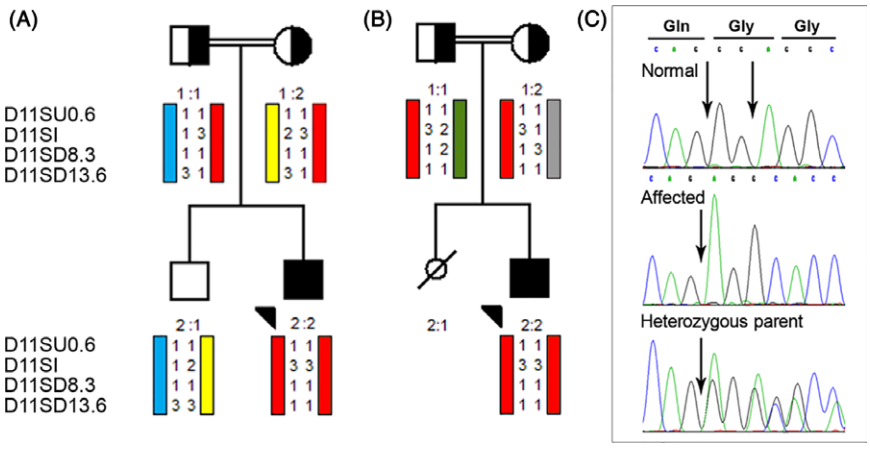

FIGURE 3 Pedigrees and mutation confirmation A, Family pedigree for the patient 1. B, Family pedigree for the patient 2. C, DNA Sanger sequencing confirmation for c.733-734delGG (p.G245Rfs*39) mutation in the index cases with homozygote condition (middle) and unaffected heterozygote parents (lower)

Institute of Iran). We acknowledge the efforts of referring clinicians. We are indebted to the patients and family members for their participation.

CONFLICT OF INTEREST

Authors declare no Conflict of Interests for this article.

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How to cite this article: Amirian A, Zafari Z, Dalili M, et al. Detection of a new KCNQ1 frameshift mutation associated with Jervell and Lange-Nielsen syndrome in 2 Iranian families. *J Arrhythmia*. 2018;34:286–290. https://doi.org/10.1002/joa3.12042